*Journal of Physiology* (1990), **426**, *pp.* 355–368
*With 5 figures*
*Printed in Great Britain*



# RESPIRATORY-RELATED DISCHARGE PATTERN OF SYMPATHETIC NERVE ACTIVITY IN THE SPONTANEOUSLY HYPERTENSIVE RAT

BY MARIA F. CZYZYK-KRZESKA AND ANDRZEJ TRZEBSKI

*From the Department of Physiology, Warsaw Medical Academy, 00-325 Warsaw, Krakowskie Przedmiescie 26/28, Poland*

(*Received 2 February 1990*)

SUMMARY

1. Synchronization of spontaneous sympathetic discharge during the respiratory cycle was studied in the cervical and renal nerves of vagotomized, normotensive Wistar–Kyoto rats (WKYs) and age-matched spontaneously hypertensive rats (SHRs). Phrenic nerve discharge was used as an index of central inspiratory activity.

2. In normotensive Wistar–Kyoto rats depression of sympathetic activity appeared at the onset of inspiration reaching a minimum at mid-inspiration. Peak maximal sympathetic discharge corresponded to postinspiratory phase; a second increase sometimes appeared in late expiration. Variations of respiratory frequency over wide range of experimental conditions by hypoxia, hyperoxia, hyper- or hypocapnia and transection of carotid sinus nerves did not affect this pattern.

3. In SHRs the respiratory-phase-related timing of sympathetic discharge was variable. In normoxia, the maximal sympathetic activity occurred in late inspiration, preceded by short depression at early inspiration and followed by postinspiratory depression. A second increase in sympathetic activity was observed in mid-expiration.

4. The pattern of respiratory phase modulated sympathetic activity in SHRs was altered by hypoxic stimulation of the peripheral chemoreceptors. The early inspiratory depression of sympathetic activity was substantially prolonged and the maximal sympathetic discharge was shifted from inspiration to early expiration. This effect was abolished after carotid sinus nerves had been cut.

5. Hypercapnic stimulation of central chemoreceptors in SHRs with carotid sinus nerves cut did not influence the timing of the sympathetic activity in relation to the respiratory phase, though the magnitude of rhythmical sympathetic discharges was increased.

6. We discuss the possibility that altered synchronization between central respiratory drive and sympathetic neuronal system may contribute to the neurogenic mechanisms of arterial hypertension in SHRs.

## INTRODUCTION

Since the original observation of respiratory modulated activity in sympathetic nerve by Adrian, Bronk & Phillips (1932), rhythmical, inspiratory-phase-related sympathetic discharge has frequently been observed in both whole-nerve and single-

fibre recordings (for references see Koepchen, Hilton & Trzebski, 1980). Several investigators have reported that these rhythmical discharges are expressed differently in various species. For instance, Eckberg, Nerhed & Wallin (1985) showed that in conscious humans maximal bursts of sympathetic activity appeared during expiration rather than inspiration, as reported previously in cats. Likewise, in Wistar rats Czyzyk, Fedorko & Trzebski (1987) found that the peak of sympathetic activity in the cervical nerve occurred during expiration. Similar expiratory-phase-related discharge in sympathetic cervical and lumbar nerves in rat was reported by Numao, Koshiya, Gilbey & Spyer (1987) and by Haselton & Guyenet (1989). Moreover, in single cervical sympathetic preganglionic neurones in rat, Gilbey, Numao & Spyer (1986) found both inspiratory- and expiratory-related discharges, the latter being observed more frequently (see Numao *et al.* 1987). Since this temporal pattern of respiratory-phase-related sympathetic activity was not influenced by bilateral vagotomy, carotid sinus nerve section or variation of respiratory frequencies over a wide range of experimental conditions it was concluded that its origin was central.

Spontaneously hypertensive rats (SHRs), an experimental model for studies of genetically controlled hypertension (Okamoto & Aoki, 1963), are characterized by exaggerated sympathetic discharges in pre- and postganglionic sympathetic nerves (Iriuchijma, 1973; Judy, Watanabe, Henry, Besch, Murphy & Hockel, 1976; Thoren & Ricksten, 1979). They also exhibit augmented respiratory drive, due to increased arterial chemoreceptor activity (Przybylski, Trzebski, Czyzewski & Jodkowski, 1982; Fukuda, Sato & Trzebski, 1987). Therefore the question arises whether respiratory–sympathetic synchronization may contribute to the increased sympathetic tone in SHRs.

The present study was undertaken to examine the respiratory synchronization of sympathetic discharge in the preganglionic cervical and postganglionic renal nerves in SHRs and in normotensive Wistar–Kyoto rats (WKYs). In addition, we studied respiratory-synchronized discharge during systemic stimulation of the peripheral and central chemoreceptors by hypoxia and hypercapnia, respectively.

Preliminary accounts of those results have been published in abstract form (Czyzyk-Krzeska & Trzebski, 1987, 1988).

## METHODS

Experiments were carried out on forty male spontaneously hypertensive rats (SHRs) and forty male normotensive Wistar–Kyoto rats (WKYs) at the age of 12–16 weeks (250–350 g). Anaesthesia was induced by inhalation of ether which in most experiments was preceded (30 min) by injection of atropine (5 mg/kg I.M.; Atropinum Sulfuricum, Polfa). Following this, a mixture of urethane (500 mg/kg) and $\alpha$-chloralose (50 mg/kg) was administered intravenously, supplemented after 4–5 h with 30–50 % of the initial dose. The adequacy of anaesthesia was determined by measuring the reaction of the animal to pinching the hindpaw. The trachea was cannulated low in the neck and catheters were placed in the femoral artery and vein in order to monitor arterial blood pressure and administer drugs, respectively. Rectal temperature was maintained between 36–38 °C with a heating blanket controlled by a feedback circuit.

The experiments were performed on paralysed and artificially ventilated animals. For paralysis, an initial dose of 200 $\mu$g/kg of pancuronium bromide (Pavulon, Organon Hesse) was used, followed by maintenance doses 200 $\mu$g/kg every 3 h. In control conditions the respiratory pump rate was set at 90–95 cycles/min; tracheal pressure did not exceed 5–6 $cmH_2O$. Animals were ventilated with room air enriched with $O_2$. A pneumothorax was performed and a positive end-expiratory pressure of 0·5–1 $cmH_2O$ was applied to prevent lung atelectasis. The animals were ventilated with gas

mixtures of 100% oxygen for chemical inactivation of arterial chemoreceptors; 12% $O_2$ in $N_2$ for stimulation of arterial chemoreceptors; 5% $CO_2$ in $O_2$ for stimulation of central chemoreceptors. Hypocapnia was produced by increasing respiratory pump rate. During exposure to hypoxia, care was taken not to reduce the $P_{a,O_2}$ value below 50 mmHg.

Blood gases were measured with a pH/blood gas analyser (205 Plastomed). During artificial ventilation arterial $O_2$ partial pressure ($P_{a,O_2}$) was maintained between 100–130 mmHg and arterial $CO_2$ partial pressure ($P_{a,CO_2}$) between 35–45 mmHg. Blood samples of 0·1–0·2 ml taken from the femoral artery were replaced with the same amount of donor blood or plasma. During ventilation with experimental gas mixtures, $P_{a,O_2}$ and $P_{a,CO_2}$ were measured during the last minute of exposure.

Control value of blood pressure for SHRs was 210/145 mmHg (S.E.M.: ±4, ±6) and for WKYs 128/84 mmHg (S.E.M.: ±5, ±2), $P < 0.001$. These values were obtained 30 min after tracheostomy and the onset of artificial ventilation.

All animals were vagotomized in order to abolish the effect of lung inflation on central respiratory activity and sympathetic preganglionic neurones (Lipski, Coote & Trzebski, 1977). Aortic nerves were cut bilaterally. In the experiments where denervation of arterial chemoreceptors was performed, the carotid sinus nerves were cut after visual identification. Denervation was verified by lack of phrenic nerve response to a short inhalation of pure nitrogen.

*Nerve recordings*

Following a mid-dorsal incision and retraction of the scapula, the cervical trunk and phrenic nerve were isolated, cut and their central ends placed on bipolar platinum electrodes for recording nerve activity. The postganglionic renal nerve was identified by the retroperitoneal approach at its entrance to the kidney hilum. All nerves were kept under warm paraffin oil in a pool made of skin flaps. After amplification (bandpass 50 Hz–5 kHz) the activity of the phrenic nerve was rectified and integrated with an RC circuit (time constant of 0·1–0·2 s). Sympathetic nerve activity was integrated by the method of moving time averaging (bandpass 50 Hz–2 kHz, time constant 0·2–1·0 s). The level of 'zero' activity of sympathetic nerves was determined by recording from the nerves after the animal had been killed with an overdose of Nembutal.

*Data collection and analyses*

Nerve activity, blood pressure and intratracheal pressure were recorded on the magnetic tape (Ampex, SP-300) and displayed on a polygraphic ink recorder or photographed from an oscilloscope (5103 N, Tektronix). Integrated spontaneous sympathetic and phrenic nerve activities were averaged over ten to sixteen respiratory cycles with a computer (Anops 101) using as a trigger a single stimulus delivered from the stimulator (Digitimer 4030) at the onset or termination of the integrated phrenic nerve burst. Intervals between phrenic bursts were accepted as the duration of expiratory phase ($T_E$). The point at which phrenic activity had decayed to the one-third of its peak value was to mark the onset of expiration. Inspiration ($T_I$) lasted from the onset of phrenic burst to the point at which expiration begun ($\frac{1}{3}$ decay of phrenic activity). The timing of the integrated and averaged sympathetic activity over the respiratory cycle was analysed quantitatively. The intervals were measured between the onset of inspiratory phase and the point of inspiratory minimal sympathetic activity ($P_{\min SA}$) and between the onset of expiration and the point of maximal sympathetic activity ($P_{\max SA}$). In case the minimal or maximal sympathetic activity reached a plateau instead of a point, the interval was measured to the beginning of the plateau.

Means (±standard errors of the mean, S.E.M.) are given for each group. Significance of the differences was calculated by one of two formulae of Student's $t$ test chosen on the basis of similarity of dispersion curves of different sets of data evaluated by Snedecor's $F$ test. $P < 0.05$ was accepted as significant for all tests used.

## RESULTS

### *Respiratory synchronization of sympathetic discharge in intact and chemodenervated normotensive Wistar–Kyoto rats (WKYs)*

Figure 1*A* shows an example of the pattern of respiratory synchronized sympathetic discharges in the cervical and renal nerves characterizing the normotensive WKY rats with the carotid chemoreceptors intact. Both nerves

exhibited essentially the same pattern. Sympathetic activity decreased with onset of inspiration, as indicated by phrenic nerve discharge, and dropped to a minimal level (point of minimal sympathetic activity) in mid-inspiration (see Table 1). Then it increased slowly during inspiratory–expiratory (I–E) transition towards a maximal

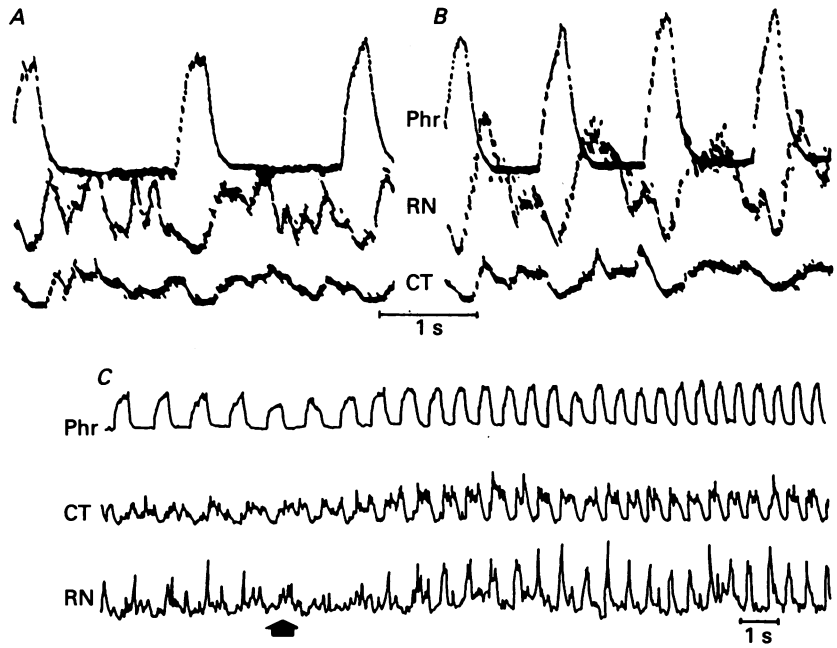


Fig. 1. Respiratory synchronization of integrated cervical trunk (CT) and renal nerve (RN) activities in normotensive WKY rat with intact carotid sinus nerves. *A*, during normoxia (averaged 16 times). *B*, during hypoxia (averaged 10 times). In both *A* and *B* averagings were triggered by onset of inspiration. *C*, arrow marks onset of hypoxia. Phr, integrated phrenic nerve activity.

level (point of maximal sympathetic activity) in the first one-third of the expiratory period (see Table 1). During late expiration, sympathetic activity diminished, but stayed above the minimal level.

Respiratory and sympathetic response to simulation of arterial chemoreceptors by hypoxia ($P_{a,O_2}$ = 50–60 mmHg) is shown in Fig. 1*B* and *C*. Respiratory activity was augmented due to an increase in both respiratory rate (see Table 1) and amplitude of the integrated burst of phrenic nerve activity ($+27 \pm 1$ %, $P < 0{\cdot}001$). Hypoxia augmented the magnitude of expiratory-phase-related sympathetic discharges (Fig. 1*B* and *C*), but the pattern of the respiratory-modulated sympathetic discharge did not change (Fig. 1*B*). The points of minimal and maximal sympathetic activity occurred during inspiration and expiration, respectively. Table 1 shows the statistical evaluation of these data. Only the interval between the point of minimal sympathetic activity and the onset of inspiration shortened in absolute values ($P < 0{\cdot}05$). During hypoxic stimulation the early inspiratory depression of sympathetic activity was in

TABLE 1. Values characterizing respiration and sympathetic activity within a respiratory cycle in WKYs and SHRs with intact carotid sinus nerves in normoxia and hypoxia and after denervation of carotid chemoreceptors

| | $f$ | $T_I$ (s) | $T_E$ (s) | Nerves | $n$ | $P_{min\ SA}$ (s) | $P_{max\ SA}$ (s) |
|---|---|---|---|---|---|---|---|
| WKYs | | | | | | | |
| Chemointact control | 35±2 | 0·54±0·05 | 1·25±0·06 | CT | 13 | 0·33±0·04 | 0·42±0·07 |
| | | | | RN | 7 | 0·29±0·03 | 0·33±0·08 |
| Chemointact hypoxia | 53±4 *** | 0·43±0·07 | 0·73±0·07 *** | CT | 6 | 0·22±0·04* | 0·31±0·05 |
| | | | | RN | 4 | 0·17±0·02* | 0·29±0·05 |
| Chemodenervated | 38±2 | 0·45±0·05 | 1·17±0·07 | CT | 15 | 0·26±0·02 | 0·35±0·05 |
| | | | | RN | 6 | 0·22±0·03 | 0·44±0·09 |
| SHRs | | | | | | | |
| Chemointact control | 38±2 | 0·46±0·05 | 1·10±0·05 | CT | 8 | 0·13±0·04 | 0·07±0·02 |
| | | | | RN | 2 | 0·09±0·03 | 0·03±0·02 |
| Chemointact hypoxia | 52±5 *** | 0·33±0·02 ** | 0·87±0·11 * | CT | 6 | 0·26±0·06** | 0·26±0·06*** |
| | | | | RN | 2 | 0·20±0·07 | 0·30±0·05 |
| Chemo-denervated | 37±1 | 0·50±0·03 | 1·17±0·07 | CT | 26 | 0·13±0·02 | −0·02±0·02* |
| | | | | RN | 9 | 0·04±0·03 | −0·05±0·06 |

*$P \leqslant 0·05$, **$P \leqslant 0·01$, ***$P \leqslant 0·001$.

$f$, respiratory frequency; $T_I$, inspiratory time; $T_E$, expiratory time; $P_{min\ SA}$, the interval between onset of inspiration and early inspiratory point of minimal sympathetic activity; $P_{max\ SA}$, the interval between the onset of expiration and the point of maximal sympathetic activity; CT and RN, cervical trunk and renal nerve, respectively; $n$, number of experiments.

some cases slightly prolonged over the period of inspiration. Hypoxia caused a decrease in blood pressure to 106/70 (S.E.M.: ±5, ±3) mmHg.

The respiratory synchronization of sympathetic discharge in a normotensive rat in which the carotid sinus nerves had been cut is shown in Fig. 2*A*. The inspiratory

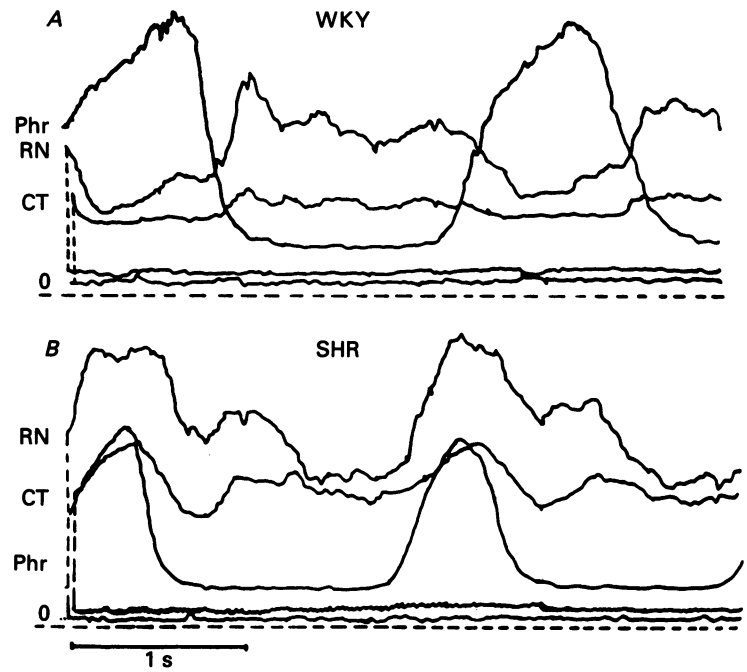


Fig. 2. Pattern of the integrated and averaged (16 times) cervical trunk (CT) and renal nerve (RN) activities in chemodenervated, paralysed and artificially ventilated normotensive WKY rat (*A*) and spontaneously hypertensive rat (*B*); averaging triggered by onset of inspiration. Horizontal dashed lines, baseline of the computer. 0, averaged and integrated signal recorded from sympathetic nerves after animals had been killed. The same amplification of activities of both sympathetic nerves in both rats was used. Time scale is for *A* and *B*.

depression and early expiratory facilitation of sympathetic discharges were the same as those observed in intact animals (compare with Fig. 1*A*). The delays of the points of minimal and maximal sympathetic activity were not different from those measured in intact animals (see Table 1). Hypercapnia ($P_{a,CO_2}$ = 55–60 mmHg) augmented the magnitude of expiratory-phase-related rhythmical sympathetic discharges but did not change the absolute timing of their relationship during the respiratory cycle, as has been described earlier (Czyzyk *et al.* 1987). Administration of hypercapnic gas mixture (5% $CO_2$ in $O_2$) caused an increase in blood pressure to 166/109 (S.E.M.: ±8, ±5) mmHg. Hypocapnia ($P_{a,CO_2}$ = 15–25 mmHg) had no effect on the temporal pattern of respiratory-related synchronization; although the magnitude of the rhythmical sympathetic discharges decreased in parallel with the amplitude of phrenic bursts.

*Respiratory synchronization of sympathetic discharges in intact spontaneously hypertensive rats (SHRs)*

Figure 3*A* and *Ca* shows examples of the pattern of respiratory-phase-related sympathetic activity during normoxia in artificially ventilated rats in which $P_{a,CO_2}$

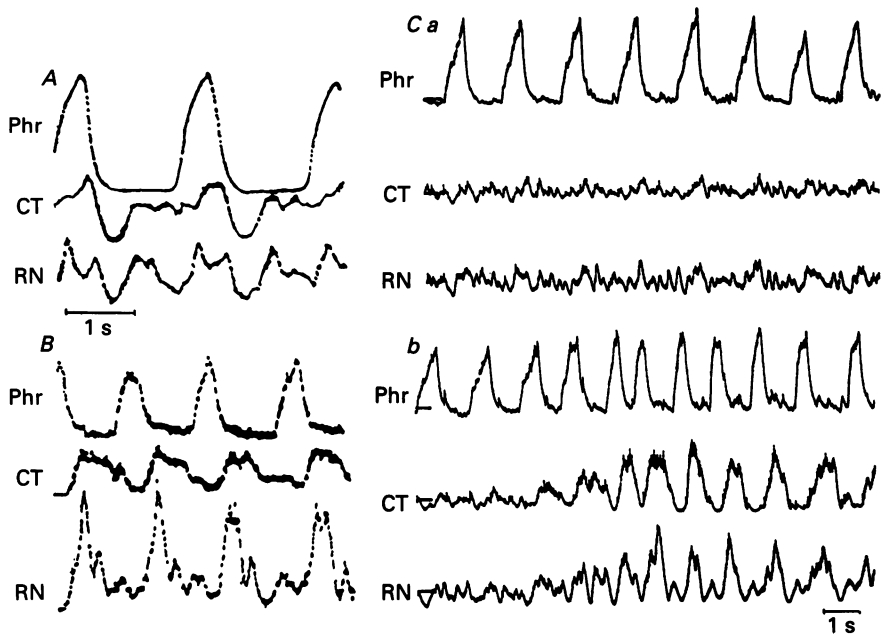


Fig. 3. Respiratory-modulated integrated cervical trunk (CT) and renal nerve (RN) activities in SHR. *A*, during normoxia (averaged 16 times); *B*, during hypoxia (averaged 8 times); averaging was triggered by onset of inspiration. *Ca* and *b*, effect of hypoxia on integrated sympathetic and phrenic (Phr) activities in another SHR. *a*, control; *b*, after hypoxia had been applied. The horizontal bars at the beginning of each trace are drawn at the same arbitrary chosen level for each trace in both *a* and *b*. Time scales are for *A* and *B* and *Ca* and *b* respectively.

was maintained between 40–50 mmHg. In normoxia ($P_{a,O_2}$ = 90–105 mmHg), sympathetic activity decreased slightly at the onset of inspiration, then augmented to the point of maximal activity at the end of inspiration or at the start of expiration. Both points of minimal and maximal sympathetic activity appeared substantially earlier within the respiratory cycle than in normotensive rats under the same experimental conditions (see Table 1). Sympathetic activity reached the second minimum at the beginning of expiration and then increased above this minimal level in late expiration. This early expiratory depression of sympathetic activity in SHRs corresponded to the period of the respiratory cycle in which sympathetic discharge in normotensive rats was maximal. There were no differences in this pattern of respiratory modulated sympathetic activity in either the renal or cervical nerves (Fig. 3*A*).

The response of the phrenic nerve and both sympathetic nerves to hypoxic stimulation of arterial chemoreceptors is shown on Fig. 3*B* and *Cb*. Systemic hypoxia produced a significant ($P < 0.001$) increase in respiratory rate (see Table 1) but failed to augment the amplitude of phrenic discharge. In fact, a slight decrease

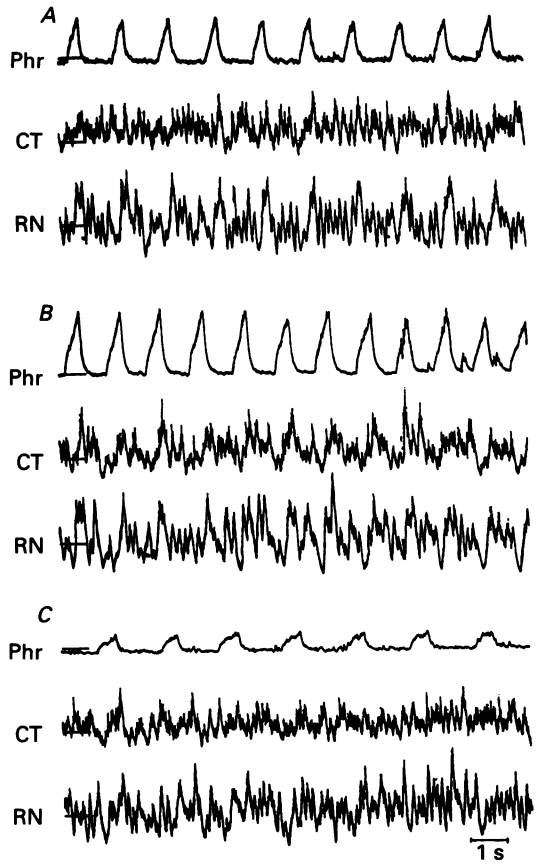


Fig. 4. Effects of $CO_2$ on integrated cervical (CT), renal (RN) and phrenic (Phr) nerve activities in SHR. *A*, normocapnia; *B*, hypercapnia; *C*, hypocapnia. Horizontal bars at the onset of each trace mark the same arbitrary chosen level of activity on respective traces. Time scale is for *A*, *B* and *C*.

in the magnitude of phrenic burst was observed ($-2 \pm 0.4$ %). Simultaneously, the postinspiratory phrenic after-discharge were enhanced. Hypoxia decreased blood pressure to 155/97 (S.E.M.: $\pm 8$, $\pm 3$) mmHg.

In hypoxic conditions the tonic level of sympathetic output did not change (Fig. 3*Ca* and *b*) and the increase in the nerve activity was mainly due to enhanced respiratory-related activity (Fig. 3*Cb*). However, the pattern of respiratory-cycle-synchronized discharges was different from control (compare panels *A* and *B* of Fig. 3). The early inspiratory decrease of sympathetic activity was substantially enhanced (Fig. 3*B*), as expressed by a significant ($P < 0.01$) prolongation of the interval

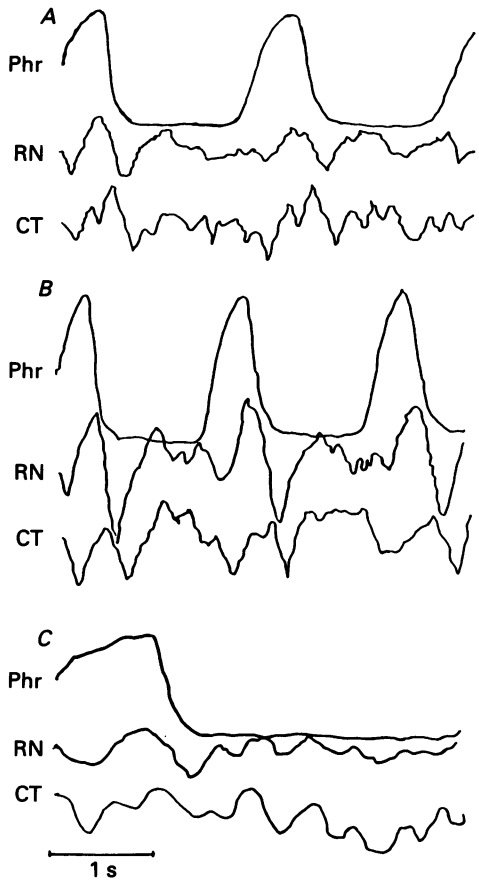


Fig. 5. Effects of $CO_2$ on the pattern of respiratory modulation of sympathetic activity in renal nerve (RN) and cervical trunk (CT) of a different SHR to that of Fig. 4. Activities of all nerves were integrated and averaged 16 times, averaging was triggered by onset of inspiration. *A*, normocapnia; *B*, hypercapnia; *C*, hypocapnia. Time scale is for *A*, *B* and *C*.

between the onset of inspiration and the point of minimal sympathetic activity (see Table 1). The increase of sympathetic activity appeared in expiration (Fig. 3*B* and *Cb*) and the point of maximal sympathetic activity occurred during postinspiration. The interval between the onset of expiration and the point of maximal sympathetic activity was significantly prolonged ($P < 0{\cdot}001$) (see Table 1). The early expiratory depression of sympathetic activity became less prominent or disappeared. Therefore, in hypoxaemia the pattern of respiratory synchronized discharges in SHRs was essentially the same as in normotensive rats. However, after both carotid sinus nerves had been cut hypoxia failed to change the pattern of respiratory timing of sympathetic discharges in SHRs.

*Respiratory synchronization of sympathetic discharges in chemodenervated spontaneously hypertensive rats (SHRs)*

Figure 2*B* shows the central pattern of respiratory modulation of sympathetic discharges in vagotomized, normoxic SHRs after both carotid sinus nerves had been cut. The respiratory modulation of sympathetic discharges was substantially similar to the normoxic, normocapnic SHRs with carotid sinus nerves left intact. The early inspiratory point of minimal sympathetic activity was less apparent than in intact SHRs, but in the cases when it was present, its delay from the beginning of inspiration was not different from intact, control animals (see Table 1). The point of maximal sympathetic activity occurred significantly earlier ($P < 0{\cdot}05$) in the respiratory cycle, but consistently still in late inspiration rather then at the inspiratory–expiratory switch point (see Table 1). The other points characterizing rhythmical changes of sympathetic activity within the respiratory cycle were not noticeably influenced by carotid body chemodenervation. Neither an increase nor a lowering of systemic arterial pressure, by blood infusion or withdrawal, affected the pattern of sympatho-respiratory synchronization.

Figure 4 demonstrates the integrated responses of phrenic, cervical and renal nerves to the changes in $P_{a,CO_2}$. Systemic hypercapnia ($P_{a,CO_2} = 45$–$55$ mmHg) increased and systemic hypocapnia ($P_{a,CO_2} = 30$–$20$ mmHg) decreased the magnitude of both phrenic and sympathetic discharges. Systemic hypercapnia increased mainly the late inspiratory peak and early expiratory depression rather then the mean level of activity (compare panels *B* and *A* of Fig. 4). The pattern of timing of sympathetic discharge during the respiratory cycle has not been influenced by hypercapnia (Fig. 5*A* and *B*). Administration of a hypercapnic gas mixture increased blood pressure to 233/175 mmHg (S.E.M.: $\pm 5$, $\pm 3$). Systemic hypocapnia ($P_{a,CO_2} =$ 20–35 mmHg) decreased respiratory modulation of sympathetic activity (compare panels *C* and *A* of Fig. 4). No alteration in the temporal pattern of respiratory-phase-modulated sympathetic activity was observed (Fig. 5*C*).

## DISCUSSION

The present results showed that spontaneously hypertensive rats exhibited a different pattern of respiratory-modulated sympathetic discharges than normotensive Wistar–Kyoto rats. Systemic hypoxia and hypercapnia, sufficient to evoke respiratory response in the phrenic nerve, increased sympathetic activity by influencing the magnitude of respiratory-related discharges rather than the tonic activity. Additionally, hypoxia altered the temporal synchronization of sympathetic discharge during the respiratory cycle in SHRs but not in WKYs. Changes in arterial $P_{CO_2}$ did not affect the timing of respiratory synchronization of sympathetic activity in either strain.

Dissimilar patterns of respiratory–sympathetic synchronization in SHRs and WKYs were of central origin, since they occurred in animals in which the peripheral mechano- and chemoreceptors had been denervated. Moreover the different respiratory–sympathetic synchronization in WKYs and SHRs cannot be explained by different resting blood pressure because changes of the latter in each strain of rats

did not affect the pattern of respiratory modulation of sympathetic activity. The shift of maximal sympathetic discharge from one respiratory phase to another in response to hypoxia was due to arterial chemoreceptor-mediated reflexes, since it disappeared after the carotid sinus nerves had been cut. Similarly, denervation of chemoreceptors abolished the sympathoexcitatory effect of hypoxia in rats (Fukuda, Sato, Suzuki & Trzebski, 1989). However, as carotid sinus nerves also contain baroreceptors fibres the possibility exists that altered baroreceptor input, due to lowered blood pressure during hypoxia, might influence the timing of sympathetic discharge in the respiratory cycle. This possibility seems improbable as hypovolaemic lowering of blood pressure did not produce this effect. Recently, it has been reported that the baroreceptor reflex did not modify the central respiratory modulation of sympathetic output in rats (Haselton & Guyenet, 1989). Changes in $CO_2$ levels, in peripherally chemodenervated rats, affected sympathetic output apparently through the central chemoreceptors. Consistent with what has been reported earlier in cats (Millhorn, 1986), $CO_2$ level influenced the magnitude of respiratory-related discharge without affecting the mean level of sympathetic activity or its respiratory-related pattern.

This report is the first to examine the respiratory modulation of sympathetic activity in spontaneously hypertensive rats. It is tempting to suggest that altered respiratory modulation of sympathetic activity in SHRs may cause greater and/or more functionally efficient sympathetic output during each respiratory cycle. This could possibly participate in the mechanism of increased sympathetic tone and in pathogenesis of their arterial hypertension. In these experiments, Wistar–Kyoto rats were used as control for spontaneously hypertensive rats. This strain of rats represents a standard genetic control for SHRs. It had also been shown that Wistar rats, another normotensive strain, exhibited the same pattern of respiratory modulation of sympathetic activity in both nerves examined as WKYs (Czyzyk *et al.* 1987; Czyzyk-Krzeska, 1988). However, in order to estimate fully the role of altered respiratory-synchronized discharges in primary hypertension of SHRs, additional studies of other sympathetic outputs are necessary, as other patterns of respiratory modulation of sympathetic activity have been reported in some sympathetic nerves in Sprague–Dawley normotensive rats (Numao *et al.* 1987).

The mechanism causing the difference in the pattern of respiratory modulation of sympathetic discharge between SHRs and WKYs, as well as among various species remains to be determined. Gilbey *et al.* (1986) showed that rats' sympathetic preganglionic neurones projecting to the cervical trunk exhibited expiratory-, inspiratory- and inspiratory–expiratory-related activity. This last pattern of firing was very similar to the type of sympathetic nervous discharges observed in SHRs. Respiratory-phase-related firing has also been observed in the supraspinal rostro-ventrolateral medullary neurones in the rat; most of the neurones exhibiting augmented activity during expiration, associated with expiratory-related discharge in lumbar sympathetic trunk, but some were active during inspiration or in postinspiratory phase (Haselton & Guyenet, 1989). Therefore, the respiratory–sympathetic coupling in rats is present even at the level of neurones antecedent to sympathetic preganglionic neurones (Haselton & Guyenet, 1989). In this regard, the dorsal neurones within rostral para-ambigual area were shown to determine temporal

pattern of respiratory–sympathetic synchronization in rats (Baradziej & Trzebski, 1989). Similarly, the respiratory-phase-related discharge has also been reported in sympathetic premotoneurones in the ventrolateral medulla of the cat (McAllen, 1987). All those data indicate that, in rats, sympathetic neurones exhibit some variability of respiratory-coupled patterns of discharge and the prevalance of different neuronal populations may account for the differences between SHRs and normotensive rats.

The pattern of inspiratory-phase-related sympathetic discharge in SHRs shows some characteristics described earlier in cats. Bainton, Richter, Seller, Ballantyne & Klein (1985) distinguished early inspiratory and postinspiratory depression of sympathetic activity in the cat. These authors suggested further that a slight and transient early inspiratory depression in sympathetic activity in the cat depended on the early inspiratory inhibitory interneurones, whereas the strong sympathetic depression during stage I expiration could be due to postinspiratory inhibitory interneurones. The effects of postinspiratory neurones on the sympathetic activity were further supported by Lawson, Richter, Ballantyne & Lalley (1989), who showed inhibition of sympathetic activity in parallel with prolonged phrenic post-inspiratory activity and activation of postinspiratory neurones during stimulation of arterial chemoreceptors. These findings may provide partial interpretation of the type of respiratory modulation of sympathetic discharges in normoxic SHRs. However, the presumably sympathoinhibitory effect of postinspiratory interneurones cannot account for the dominating postinspiratory pattern of sympathetic discharges occurring in SHRs during stimulation of arterial chemoreceptors with hypoxia or in the normotensive rats. This phenomenon may be interpreted as a chemoreceptor-mediated sympathoexcitatory effect occurring throughout expiration as the basic level of the minimal sympathetic activity in inspiration did not change during hypoxia. In this regard it has been shown that stimulation of the carotid chemoreceptors activates expiratory neurones in cats (Lipski, Trzebski, Chodobska & Kruk, 1984). Also, according to the above-cited findings (Lawson *et al.* 1989), the enhanced postinspiratory phrenic activity in the hypoxic conditions in SHRs may indicate stimulation of the postinspiratory neurones in the chemoreceptor reflex. The possibility exists that activation of chemoreceptor afferents causes also some disfacilitatory effects on sympathetic activity during inspiration as the tonic inhibitory effects of chemoreceptor stimulation on respiratory neurones have been reported (Lawson *et al.* 1989).

Present results suggest complex connections between respiratory and pre-sympathetic neurones in the rat. Heterogenous categories of respiratory-related neurones have been reported in rat (Saether, Hilaire & Monteau, 1987), but still very little is known about the organization of the respiratory network in this species. Also the responsiveness of respiratory neuronal categories to carotid chemoreceptor stimulation and/or inactivation may vary among species and between normotensive and hypertensive rats. This possibility may be exemplified by the attenuated respiratory response to hypoxia in SHRs, when no increase in phrenic nerve burst occurred. The detailed mechanism of respiratory–sympathetic synchronization in the rat requires further investigations.

The authors express their appreciation to Dr D. E. Millhorn and for his helpful advice during the

preparation of the manuscript and to Dr E. E. Lawson for reading the manuscript. This work was supported by a Wellcome Trust Foundation grant and by Polish grant C.P.B.R. 11.6–58.3.

## REFERENCES

ADRIAN, E. D., BRONK, D. W. & PHILLIPS, G. (1932). Discharges in mammalian sympathetic nerves. *Journal of Physiology* **74**, 115–153.

BAINTON, C. R., RICHTER, D. W., SELLER, H., BALLANTYNE, D. & KLEIN, J. P. (1985). Respiratory modulation of sympathetic activity. *Journal of the Autonomic Nervous System* **12**, 77–90.

BARADZIEJ, S. & TRZEBSKI, A. (1989). Specific areas of the ventral medulla controlling sympathetic and respiratory activities and their functional synchronization in the rat. *Progress in Brain Research* **81**, 193–204.

CZYZYK, M. F., FEDORKO, L. & TRZEBSKI, A. (1987). Pattern of the respiratory modulation of the sympathetic activity is species dependent: synchronization of the sympathetic outflow over the respiratory cycle in the rat. In *Organization of the Autonomic Nervous System, Central and Peripheral Mechanisms*, ed. CIRIELLO, J., CALARESU, F. R., RENAUD, L. P. & POLOSA, C. pp. 143–152. A. R. Liss, New York.

CZYZYK-KRZESKA, M. F. (1988). Respiratory modulation of sympathetic activity in the spontaneously hypertensive rats (SHRs). Ph.D. thesis, Warsaw Medical Academy.

CZYZYK-KRZESKA, M. F. & TRZEBSKI, A. (1987). Respiratory synchronization of the sympathetic activity (SA) in spontaneously hypertensive rats (SHRs). *The Second World Congress of Neuroscience (IBRO) Budapest, Hungary* **S327**, 981 (abstract).

CZYZYK-KRZESKA, M. F. & TRZEBSKI, A. (1988). Respiratory timing of chemoreceptor sympatho-excitatory reflex is inversed in SHRs. *12th Scientific Meeting of the International Society of Hypertension, Kyoto, Japan* p. 1403 (abstract).

ECKBERG, D. L., NERHED, CH. & WALLIN, G. (1985). Respiratory modulation of the muscle sympathetic and vagal cardiac outflow in man. *Journal of Physiology* **365**, 181–196.

FUKUDA, Y., SATO, A., SUZUKI, A. & TRZEBSKI, A. (1989). Autonomic nerve and cardiovascular responses to changing blood oxygen and carbon dioxide levels in the rat. *Journal of the Autonomic Nervous System* **28**, 61–74.

FUKUDA, Y., SATO, A. & TRZEBSKI, A. (1987). Carotid chemoreceptor discharge responses to hypoxia and hypercapnia in normotensive and spontaneously hypertensive rats. *Journal of the Autonomic Nervous System* **19**, 1–11.

GILBEY, M. P., NUMAO, Y. & SPYER, K. M. (1986). Discharge patterns of cervical sympathetic preganglionic neurones related to central respiratory drive in the rat. *Journal of Physiology* **378**, 253–266.

HASELTON, J. R. & GUYENET, P. G. (1989). Central respiratory modulation of medullary sympathoexcitatory neurons in rat. *American Journal of Physiology* **256**, R739–750.

IRIUCHIJIMA, J. (1973). Sympathetic discharge rate in spontaneously hypertensive rats. *Japanese Heart Journal* **14**, 350–356.

JUDY, W. V., WATANABE, A. M., HENRY, D. P., BESCH, H. R., MURPHY, W. R. & HOCKEL, G. M. (1976). Sympathetic nerve activity. Role in regulation of blood pressure in the spontaneously hypertensive rat. *Circulation Research* **38**, suppl. 11, 21–29.

KOEPCHEN, H. P., HILTON, S. M. & TRZEBSKI, A. (1980). *Central Interaction between Respiratory and Cardiovascular Control Systems*. Springer Verlag, Berlin, Heidelberg, New York.

LAWSON, E. E., RITCHER, D. W., BALLANTYNE, D. & LALLEY, P. (1989). Peripheral chemoreceptor inputs to medullary inspiratory and postinspiratory neurons in cats. *Pflügers Archiv* **414**, 523–533.

LIPSKI, J., COOTE, J. H. & TRZEBSKI, A. (1977). Temporal pattern of antidromic invasion latencies of sympathetic preganglionic neurons related to central inspiratory activity and pulmonary stretch receptor reflex. *Brain Research* **135**, 162–166.

LIPSKI, J., TRZEBSKI, A., CHODOBSKA, J. & KRUK, P. (1984). Effects of carotid chemoreceptor excitation on medullary expiratory neurons in cats. *Respiration Physiology* **57**, 279–291.

MCALLEN, R. M. (1987). Central respiratory modulation of subretrofacial neurons in the cat. *Journal of Physiology* **388**, 533–545.

MILLHORN, D. E. (1986). Neural respiratory and circulatory interaction during chemoreceptor stimulation and cooling of ventral medulla in cats. *Journal of Physiology* **370**, 217–231.

NUMAO, Y., KOSHIYA, N., GILBEY, M. P. & SPYER, K. M. (1987). Central respiratory drive-related activity in sympathetic nerves of the rat: the regional difference. *Neuroscience Letters* **81**, 279–284.

OKAMOTO, K. & AOKI, K. (1963). Development of a strain of spontaneously hypertensive rats. *Japanese Circulation of Journal* **27**, 282–293.

PRZYBYLSKI, J., TRZEBSKI, A., CZYZEWSKI, T. & JODKOWSKI, J. (1982). Responses to hyperoxia, hypoxia, hypercapnia and almitrine in spontaneously hypertensive rats. *Bulletin of European Physiopathological Respiration* **18**, suppl. 4, 145–154.

SAETHER, K., HILAIRE, G. & MONTEAU, R. (1987). Dorsal and ventral respiratory groups of neurons in the medulla of the rat. *Brain Research* **419**, 87–96.

THOREN, P. & RICKSTEN, S. E. (1979). Recordings of renal and splanchnic sympathetic nervous activity in normotensive and spontaneously hypertensive rats. *Clinical Sciences* **57**, 197–199s.